Structure of Natural Killer (NK) Cell Receptors that Recognize Class I MHC Molecules

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Abstract No. Boyi1392
Beamline(s): **X9B**

Introduction: Natural killer (NK) cells constitute a vital part of the innate immune system. Like cytotoxic T-cells, NK cells express surface receptors that interact with polymorphic class I MHC molecules and regulate cell lysis. In contrast to T-cell receptor (TCR)/MHC mediated cellular activation, recognition of class I molecules by NK cells can result in either target cell lysis or the inhibition of lysis depending upon whether the receptor contains a charged transmembrane residue that interacts with the ITAM containing DAP-12 or a cytoplasmic ITIM motif. Furthermore, MHC recognition by NK cell receptors is less allele specific and less peptide dependent. Of particular interest are inhibitory NK cell receptors that protect target cells bearing certain class I MHC allotypes from NK-mediated lysis. Cells that have lost class I MHC expression such as certain tumor or virus infected cells will not be engaged by inhibitory receptors and consequently are susceptible to NK cell lysis. The two structurally distinct superfamilies of NK cell receptors are the immunoglobulin (Ig)-like and C-type lectin like receptors (CTLR). To date the crystal structures of three killer cell Ig-like (KIR) receptors, KIR2DL1, 2DL2 and 2DL3 and two CTLR receptors, human CD94 and murine Ly49A in complex with H-2D^d, have been determined.

Methods and Materials: The structures of HLA-Cw3 and both KIR2DL2 molecules were determined by molecular replacement using the program AmoRe. The polyalanine version of HLA-A2 (PDB entry 1B0G) was used as the model in rotation and translation searches between 10 and 3.5 Å. This yielded a clear solution with a correlation coefficient of 40.0% and an R factor of 53.8%. After rigid body refinement of individual domains using the program CNS, most side chains of HLA-Cw3 had clear electron density into which all but 40 side chains were built. A solution for the first KIR2DL2 molecule was identified using a polyalanine model of the unbound KIR2DL2 (PDB entry 2DL2) as a search model. The HLA-Cw3 position was fixed during the translation search resulting in a strong peak with a correlation coefficient of 44.1% and an R factor of 51.0%. After preliminary refinement in CNS, a second KIR molecule was clearly visible in F_o-F_c maps contoured to 2.0σ. This second KIR was included by further searches using the fixed positions of HLA-Cw3 and the first KIR yielding a final solution with a correlation coefficient of 66.6% and an R factor of 40.1%.

Results: The structure of KIR2DL2 in complex with HLA-Cw3 and the peptide GAVDPLLAL (GAV) was determined by molecular replacement methods and refined to 3.0 Å resolution. The final R factors are 23.1% and 29.4% for R_{cryst} and R_{free}, respectively. Each asymmetric unit contains two KIR (KIR_A and KIR_B) and one HLA-Cw3 molecule. The electron density is continuous in the final $2F_o$-F_c map throughout the complex except for four KIR surface loops (residues 143-145 of KIR_A and residues 58, 121 and 158-159 of KIR_B) located in regions away from the HLA interface. Only KIR_A interacts with HLA-Cw3. KIR_B is situated on the distal end of the D2 domain of KIR_A, providing lattice contacts between symmetry related receptors in the crystal. Thus the stoichiometry of KIR/HLA binding is 1:1 in the crystal. This is also supported by analytical equilibrium centrifugation experiments in which the K_D for KIR2DL2 and HLA-Cw3 association was measured as 17 μm and sedimentation curves were best described by a 1:1 interaction model.

KIR_A interacts with HLA-Cw3 through surface loops near its interdomain hinge region. The binding site for KIR is located toward the C-terminal end of the peptide and the corresponding region of the α1 and α2 helices. The orientation of the KIR is such that its N-terminal D1 domain interacts with polymorphic regions of the α1 helix, residues 69-84, and its D2 domain interacts with more conserved regions of the α2 helix, residues 145-151. This produces a nearly orthogonal (88º) docking of KIR to its class I ligand and permits direct contact between KIR and residues 7 and 8 of the GAV peptide.

Conclusions: KIR binds in a nearly orthogonal orientation across the α1 and α2 helices of Cw3 directly contacting positions 7 and 8 of the peptide. No significant conformational changes in KIR are observed upon complex formation. The receptor footprint on HLA overlaps with, but is distinct from that of the T-cell receptor. Charge complementarity dominates the KIR/HLA interface and mutations disrupting interface salt bridges seriously diminished binding. While most KIR contacts are to conserve HLA-C residues, a hydrogen bond between Lys 44 of KIR2DL2 and Asn 80 of Cw3 confers the allotype specificity. KIR contact restricts P8 of the peptide to residues smaller than Val. A second KIR/HLA interface produced an ordered receptor-ligand aggregation in the crystal, which may resemble receptor clustering during immune synapse formation.

Acknowledgments: We thank Z. Dauter and C. Titlow for help with synchrotron data collection; J. Lukszo for peptide synthesis; C. Hammer for mass spectroscopy measurements; M. Garfield for N-terminal amino acid sequencing; A. Winterhalter and N Mifsud for assistance with the mutagenesis and DNA sequencing and D. Margulies for help with BIAcore SPR measurements. This work is supported by intramural research funding from the National Institute of Allergy and Infectious Diseases and by a grant from the National Health and Medical Research Council, Australia.

References: J Boyington, S Motyka, P Schuck, A Brooks and P Sun, “Crystal structure of an NK cell immunoglobulin-like receptor in complex with its class I MHC ligand”, Nature 405, 537-543, 2000.